



King Saud University
Arabian Journal of Chemistry

www.ksu.edu.sa
www.sciencedirect.com



ORIGINAL ARTICLE

Chiral stability-indicating HPLC method for analysis of arotinolol in pharmaceutical formulation and human plasma

Maha A. Sultan ^{*}, Mohamed M. Hefnawy, Mona M. Al-Shehri

Department of Pharmaceutical Chemistry, College of Pharmacy, King Saud University, P.O. Box 2457, Riyadh 11451, Saudi Arabia

Received 3 June 2009; accepted 12 September 2009

Available online 14 April 2010

KEYWORDS

Arotinolol;
Chirobiotic V;
Chiral separation;
Degradation study;
Human plasma;
Pharmaceutical formulation

Abstract An enantioselective stability-indicating high performance liquid chromatographic method was developed for the analysis of arotinolol in standard solution. The degradation behaviour of arotinolol was investigated under different stress conditions recommended by International Conference on Harmonization (ICH). Resolution of the drug and complete separation from its degradation products were successfully achieved on a Chirobiotic V column, using UV detector set at 315 nm, polar organic mobile phase (POM) consisting of methanol:glacial acetic acid:triethylamine, 100:0.02:0.03, (v/v/v), and a flow rate of 1 ml/min. The drug was subjected to oxidation, hydrolysis, photolysis, and heat to apply stress conditions. The drug was found to degrade in alkaline, acidic, oxidative conditions and when exposed to heat. The drug was stable to sunlight. The method reported here has also been successfully applied to pharmaceutical formulation and to human plasma that spiked with stock solutions of arotinolol enantiomers.

Arotinolol enantiomers were recovered from plasma by using liquid–liquid extraction procedure with ethyl ether. The method was highly specific, where degradation products and coformulated compounds did not interfere, and was sensitive with good precision and accuracy and was linear over the range of 50–400 ng/ml ($R^2 > 0.9981$) with a detection limit of 20 ng/ml for each enantiomer. The mean extraction efficiency for arotinolol was in the ranges 96–104% for each enantiomer. The mean relative standard deviation (RSD) of the results of within-day precision and accuracy of the drug were $\leq 7.1\%$. There was no significant difference between inter- and intra-day studies for each enantiomers which confirmed the reproducibility of the assay. The overall recoveries of arotinolol enantiomers from pharmaceutical formulations were in the ranges 97.6–101.8%.

© 2010 King Saud University. All rights reserved.

^{*} Corresponding author.

E-mail address: sultan_maha@yahoo.com (M.A. Sultan).



1. Introduction

The stability-indicating assay is a method applied to ensure the stability of a drug. The presence of impurities and degradation products can change chemical, pharmacological and toxicological properties of drugs having significant impact on their quality and safety. Since drugs are, especially, sensitive to environmental factors, strict storage conditions are

necessary (Ahuja, 1998; Ahuja and Alsante, 2003; FDA, 1998).

The ICH guideline Q1A on Stability Testing of New Drug Substances suggests that the testing of those features which are susceptible to change during storage and are likely to influence quality, safety and efficacy must be done by validated stability-indicating methods. Stress testing should be carried out on a drug to establish its inherent stability characteristics and to support the suitability of the proposed analytical method. It is also suggested that stress testing should include the effect of temperature, susceptibility across a wide range of pH values, as well as oxidative and photolytic conditions (ICH, 2003).

Like stability-indicating study, the enantioseparation has a great impact on the pharmacological and toxicological properties of chiral drugs in terms of the control of individual variability in clinical responses between the enantiomers. A prerequisite for progress in acquiring knowledge of stereospecific pharmacodynamics and pharmacokinetics of optical isomers is the development of an enantioselective analytical methodology. High performance liquid chromatography (HPLC) is one of the famous analytical techniques commonly employed in conducting stability studies (Daraghmei et al., 2001; Baaske et al., 1996) and in chiral separation (Pettersson and Persson, 1998; Török et al., 2005). HPLC has gained popularity due to its high resolution capacity, speed, sensitivity and specificity.

Separation of enantiomers by HPLC can be achieved using either chiral stationary phases or chiral additives to the mobile phase. The most common HPLC approach for resolving enantiomers involves the use of chiral stationary phases (CSPs) (Subramanian, 1994). The macrocyclic antibiotic CSPs become a very important tool for separation of a wide range of structurally different chiral compounds (Duret et al., 2000). The most useful and popular among them, are based on teicoplanin and vancomycin chiral selectors (Chirobiotic Handbook, 2004). The enantioselectivity of these chiral selectors are due to several reasons: (i) they are amphoteric (i.e. contain acidic and basic ionizable groups); (ii) they have the necessary geometry and functionalities that accentuate chiral recognition in solution; and (iii) they contain both hydrophilic and hydrophobic moieties (Ekborg-Ott et al., 1998).

A simplified approach has proven very effective for the resolution of a broad spectrum of racemate analytes. The first consideration in this direction is the structure of the analytes. If the compound has more than one functional group capable of interacting with the stationary phase and at least one of those groups is on or near the stereogenic center, then the first mobile phase choice would be the polar organic phase. Due to the strong polar groups present in the macrocyclic peptides, it was possible to convert the original mobile phase concept to 100% methanol with an acid/base added to effect selectivity. The key factor in obtaining complete resolution is still the ratio of acid to base (Aboul-Enein and Ali, 2002).

Arotinolol, 5-[2-(3-*t*-butylamino-2-hydroxypropylthio)thiazol-4-yl] thiophene-2-carboxamide, Fig. 1 is a β -blocking agent. In addition it has weak α -blocking activity. It is used in therapeutics for its antihypertensive, antiangorous and antiarrhythmic properties (Imai et al., 1995). Although it is reported that the S-(+)-enantiomer is more active than the R-(−)-enantiomer, it is clinically administered as racemic mixture (Van-Zwiezen, 1993). Several methods have been published for the chiral assay of arotinolol (Moulin et al., 1992; Hefnawy, 2002;

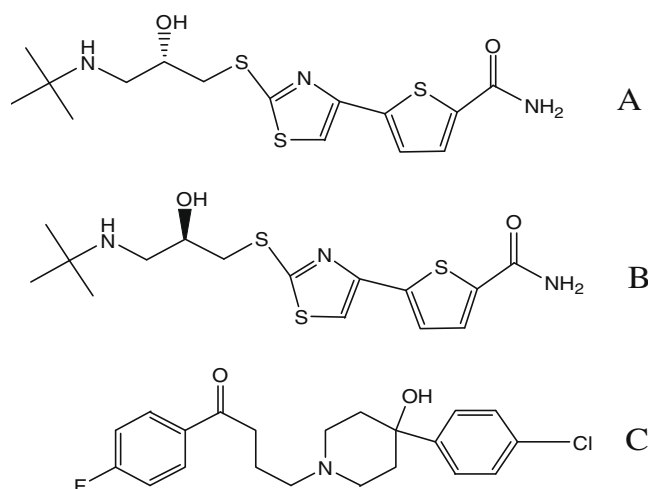


Figure 1 Chemical structures of (A) S-(+)-arotinolol, (B) R-(−)-arotinolol, and (C) haloperidol (IS).

Aboul-Enein and Hefnawy, 2003). However, a stability-indicating method for the assay of this drug is not available in the literature.

The aim of this study is to establish a validated stability-indicating HPLC method for the assay of S-(+)- and R-(−)-arotinolol in standard solution and apply this method to human plasma and a pharmaceutical formulation successfully using vancomycin CSP column as the chiral selector. None of the reported analytical methods described stability-indicating method for the chiral determination of arotinolol in presence of its degradation products. To our knowledge, this is the first report of stability-indicating method for the chiral determination of arotinolol.

2. Experimental

2.1. Chemicals and reagent

S-(+)- and R-(−)-arotinolol were gifts from Sumitomo Pharmaceutical Co. (Osaka, Japan), and haloperidol was obtained from Sigma Chemical Co. (St. Louis, MO, USA). HPLC-grade methanol, analytical grade triethylamine, glacial acetic acid and ethyl ether were purchased from BDH Chemicals (Poole, UK). Analytical grade ammonium acetate, hydrochloric acid, sodium hydroxide and hydrogen peroxide were purchased from WINLAB (UK). Deionized water was purified using a cartridge system (Picotech water system, RTP, NC, USA). Human plasma was obtained from King Khalid University hospital (Riyadh, KSA), and was kept frozen until use.

2.2. HPLC system

The HPLC instrument (Jasco, Japan) is equipped with a pump (model PU-980), a UV/vis detector (model UV-975) and injection valve with 20 μ L sample loop. Signal acquisition and data handling were performed with LG computer connected to the instrument. The CSP used in this study was the macrolide-type antibiotic vancomycin, known as Chirobiotic V (150 \times 4.6 mm i.d.) purchased from Advanced Separation Technologies (Whippany, NJ, USA). The mobile phase was methanol:glacial

acetic acid:triethylamine (100:0.02:0.03, v/v/v). The mobile phase was filtered through a Millipore membrane filter (0.2 μ m) from Nihon, Millipore (Yonezawa, Japan) and degassed before use. The flow rate was 1 ml/min and the detection wavelength (UV) was set at 315 nm.

2.3. Preparation of stock and standard solution

Stock solutions of individual S-(+)- and R-(-)-arotinolol hydrochloride were prepared in methanol to give a concentration of 80 μ g/ml. The internal standard haloperidol was prepared in methanol to give a concentration of 1 mg/ml. Appropriate dilutions of the individual stock solutions of arotinolol were made and used for constructing the calibration curves, for conducting the stability study and for spiking the plasma.

2.4. Degradation studies

All degradation studies were performed at a drug concentration of 200 ng/ml in methanol. Acid hydrolysis was performed in 0.1 and 1 M HCl at room temperature and samples were withdrawn at different time intervals. The study in alkaline condition was carried out in 0.01, 0.1 and 1 M NaOH at room temperature and samples were withdrawn at different time intervals. Oxidative study was carried out at room temperature in 3%, 10%, 20% and 30% hydrogen peroxide and samples were also withdrawn at different time intervals. Photo-degradation study was performed in methanol. The solution was exposed to sunlight for 3 days. Suitable control was kept under the dark. Additionally, thermal degradation was induced by storing the drug at 80 °C for a period of 7 days. Samples were withdrawn at appropriate time and subjected to HPLC analysis.

2.5. Preparation of plasma samples

Human plasma sample (0.5 ml) was placed into individual 1.5 ml Eppendorf tube and accurately measured aliquots of S-(+)- and R-(-)-arotinolol were added. Then 25 μ l of the internal standard solution was added to each tube and diluted with water to 1 ml and mixed well to give final concentrations of 80, 150 and 300 ng/ml of each arotinolol enantiomers. The mixture was treated with 100 μ l of 3 M ammonium acetate (pH 9) and sonicated for 5 min. The aqueous phase was extracted with 3 \times 1 ml ethyl ether. The organic phase was evaporated to dryness. The residue was dissolved in 200 μ l methanol and injected into an HPLC system. Blank human plasma samples were processed in the same manner using deionized water instead of arotinolol enantiomers.

2.6. Preparation of tablet solutions

Ten prepared tablets were grounded and powdered. An accurately weighed portion equivalent to 10 mg arotinolol was transferred to 100 ml volumetric flask diluted to the mark with methanol. The solution was sonicated for 15 min, centrifuged at 3000 rpm for 10 min. Accurately measured aliquots of the supernatant were transferred to 5 ml volumetric flasks containing 100 μ l of the internal standard and diluted to 5 ml with methanol to give final concentration of 100, 220 and 340 ng/ml of arotinolol.

2.7. Recovery

The absolute recoveries of each enantiomer from plasma were calculated by comparing drug peak area of the spiked analyte samples to unextracted analyte of stock solution that has been injected directly into an HPLC system. The assay absolute recovery for each compound, at each concentration, was computed using the following equation:

$$\text{absolute recovery} = \frac{(\text{peak area of extract} / \text{mean peak area of direct injection}) \times 100.}$$

2.8. Validation of the method

2.8.1. Linearity

Under the optimized experimental conditions, the calibration plots for the S-(-)- and R-(+)-arotinolol standard solution were prepared, the statistical evaluation of the regression parameters are shown in Table 1.

Calibration plots for the S-(-)- and R-(+)-arotinolol in plasma were prepared by diluting stock solutions with pooled human plasma to yield seven concentrations over the range of 50–400 ng/ml for each enantiomer, respectively.

Calibration standards at each concentration were extracted and analyzed in triplicate. Calibration curves of arotinolol enantiomers were constructed using the observed analyte peak area over internal standard peak area versus nominal concentrations of the analytes. Least squares linear regression analysis of the data gave slope, intercept and correlation coefficient data. From this data, a first order polynomial model was selected for each analyte.

2.8.2. Precision and accuracy

The within-run and between-run accuracy and precision of the assays in plasma were determined by assaying three QC samples in triplicate over a period of 3 days. The concentrations represented the entire range of the calibration curves. Calibration curves were prepared and analyzed daily and linear models were used to determine concentrations in the QC samples. The nine measured concentrations per concentration level (triplicates from three runs) were subjected to estimate the

Table 1 Validation parameters for the determination of arotinolol enantiomers in standard solution using the proposed method.

Parameter	S-(+)-arotinolol	R-(-)-arotinolol
Concentration range (ng/ml)	20–400	20–400
Intercept (<i>a</i>)	−0.0640	−0.0740
Slope (<i>b</i>)	0.0048	0.0049
Correlation coefficient (<i>r</i>)	0.9991	0.9996
<i>S</i> _{y/x}	0.0433	0.0194
<i>S</i> _a	0.0275	0.0124
<i>S</i> _b	0.0001	0.0001
LLQ (ng/ml) ^a	20	20
LLD (ng/ml) ^b	5	5

^a *S*/*N* = 10.

^b *S*/*N* = 3.

within-run and between-run precision. Precision was reported as % relative standard deviation (%RSD) = (S.D./mean) × 100. Percent accuracy was determined (using the data from the precision assessment) as the closeness of spiked samples to the nominal value of in-house standards. Percent accuracy was reported as %error = ((nominal concentration – measured concentration)/nominal concentration) × 100.

2.8.3. Limit of detection and limit of quantitation

The limit of detection (LOD) and the limit of quantitation (LOQ) were determined as 3 and 10 times the baseline noise, respectively, following The United States Pharmacopeia (2000). The results of the statistical analysis of the experimental data, such as the slopes, the intercepts, the correlation coefficients obtained by the linear squares treatment of the results along with standard deviation of the slope (S_b) and intercept (S_a) on the ordinate and the standard deviation of the residuals ($S_{y/x}$).

2.8.4. Selectivity

The selectivity of the assay was checked by analyzing independent blank human plasma samples and placebo tablets samples. The chromatograms of these samples were compared with chromatograms obtained by analyzing human plasma samples spiked with the analytes and chromatograms obtained by analyzing prepared tablets containing the drug, respectively. Moreover, the selectivity of the assay was checked by analyzing the stability study samples. The chromatograms of standard arotinolol solutions were compared with chromatograms obtained by analyzing the stability study samples.

3. Result and discussion

3.1. Optimization of the chromatographic condition

The complex structures of macrocyclic antibiotics are responsible for their chiral selectivity in different modes (Aboul-Enein and Ali, 2002). The possible interactions involved are π - π complexation, hydrogen bonding, inclusion complexation, dipole interactions, steric interactions, anionic and cationic bindings (Aboul-Enein and Ali, 2000). These interactions take place individually or in combination, which can result in the very high chiral recognition capacities for these antibiotics.

The chemical structures of S-(+)-(I) and R-(-)-(II) arotinolol and haloperidol (III) are shown in Fig. 1.

The polar organic mode (POM) which has been described to obtain enantioselective separation with macrocyclic antibiotic-based CSPs (Fried et al., 1998) is a good choice for enantioseparation of arotinolol. Easy-to-prepare mobile phases composed of methanol with small amounts of acetic acid (HAc) and triethylamine (TEA) were used. The baseline separation of arotinolol was achieved on the vancomycin CSP column with a polar organic mobile phase consisting of methanol:glacial acetic acid:triethylamine (100:0.02:0.03, v/v/v) (Table 2) and Fig. 2.

When using pure methanol as mobile phase, arotinolol did not elute. A small addition of HAc and TEA was sufficient to elute the enantiomers in an acceptable time. An increase in the acid and base concentrations decreases the enantioresolution values.

Table 2 Chromatographic parameter data for arotinolol enantiomers and internal standard.

Analyte	R_s^a	α^b	K^c	T_R (min) ^c
S-(+)-arotinolol	1.20	1.15	3.87 ± 0.04	9.12 ± 0.01
R-(-)-arotinolol	1.76	1.23	4.50 ± 0.07	10.26 ± 0.02
Haloperidol	^d	^d	5.52 ± 0.04	12.14 ± 0.05

^a $R_s = (t_2 - t_1)/0.5(w_2 + w_1)$.

^b Separation factor, calculated as k_2/k_1 .

^c Mean \pm SD, $n = 4$.

^d Not calculated.

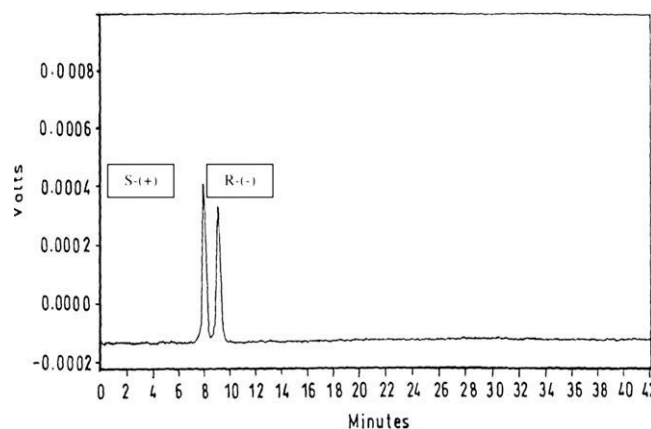


Figure 2 Chromatogram of freshly prepared arotinolol sample using Chirobiotic V column (mobile phase: methanol:glacial acetic acid:triethylamine (100:0.020:0.030, v/v/v), flow rate: 1 ml/min, UV detection: 315 nm).

3.2. Degradation studies

HPLC studies of samples obtained on stress testing of arotinolol under different conditions suggested the following degradation behaviour.

3.2.1. Acid degradation study

The drug was stable in 0.1 M HCl at room temperature for a period of 7 days. In 1 M HCl, 20.90% of arotinolol was hydrolyzed in the same period (Fig 3).

3.2.2. Alkali degradation study

It was observed that the whole drug was degraded in 1 N NaOH at room temperature after 48 h. After 7 days no degradation was observed in 0.01 N NaOH and 69.11% of the drug was degraded in 0.1 N NaOH.

3.2.3. Oxidation study

The drug was found to be highly labile to oxidation by H_2O_2 at room temperature. The reaction in 30% H_2O_2 resulted in complete degradation of the drug in 24 h. Subsequently, study was performed in 3%, 10% and 20% H_2O_2 . The whole drug was degraded after 4, 3 and 2 days, respectively.

3.2.4. Temperature study

After the drug has been exposed to 80 °C for 7 days, 35.47% of it was degraded.

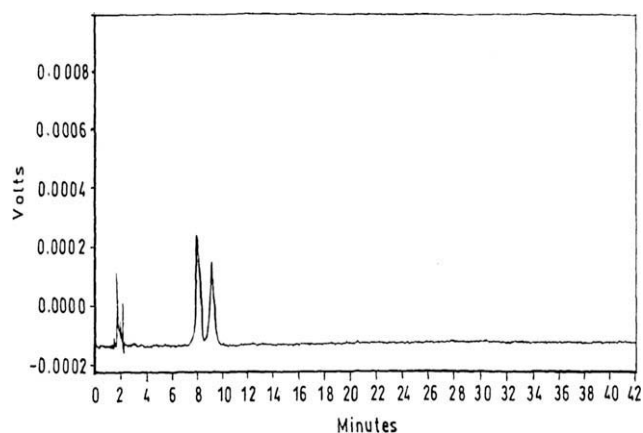


Figure 3 Chromatogram of arotinolol in 1 M HCl after a period of 7 days using Chirobiotic V column (mobile phase: methanol:glacial acetic acid:triethylamine (100:0.020:0.030, v/v/v), flow rate: 1 ml/min, UV detection: 315 nm).

3.2.5. Photolytic study

No degradation was observed of drug solution in methanol after exposure to sunlight for 3 days, indicating that light had no effect on the degradation of arotinolol solution in methanol.

The degradation products did not interfere with the drug indicating that the method is highly selective, and could not be detected in a run time of 1 h in all the degraded samples.

3.3. Applications to spiked human plasma

The extraction procedure used in this study afforded percentage recovery ranges of 96–104% for linearity in the range of 50–400 ng/ml for both enantiomers, Table 3. The addition of ammonium acetate was necessary to obtain such recoveries.

Table 3 Validation parameters for the determination of arotinolol enantiomers in human plasma using the proposed method.

Parameters	S-(+)-arotinolol	R-(–)-arotinolol
Concentration range (ng/ml)	50–400	50–400
Intercept (<i>a</i>)	–0.04003	–0.06567
Slope (<i>b</i>)	0.00159	0.00180
Correlation	0.9988	0.9971
<i>Coefficient (r)</i>		
<i>S_{y/x}</i>	0.01487	0.02505
<i>S_b</i>	0.00005	0.00009
LOQ (ng/ml)	50	50
LOD (ng/ml) ^a	20	20

^a S/N = 3.

Chromatograms of a blank human plasma sample (A) and an extracted sample from plasma (B) are in Fig. 4. It is clear that there were no interferences from endogenous plasma contents indicating the high selectivity of the proposed method.

3.4. Precision and accuracy

A summary of the accuracy and precision results in spiked human plasma is given in Table 4. The acceptance criteria (within-run and between-run %RSD of less than 15% and accuracy between 85% and 115%) were met in all cases. The within-run precision and accuracy (*n* = 3) expressed as %RSD and %error were 1.9–5.5% and 2.1–3.1%, respectively, for S-(+)-arotinolol and 1.9–7.1% and 2.2–3.4%, respectively, for R-(–)-arotinolol.

The between-run precision and accuracy (*n* = 9) expressed as %RSD and %error were 4.1–5.7% and 0.5–1.9% for S-(+)-arotinolol, respectively, and 3.8–4.8% and 2.1–3.8% for R-(–)-arotinolol, respectively.

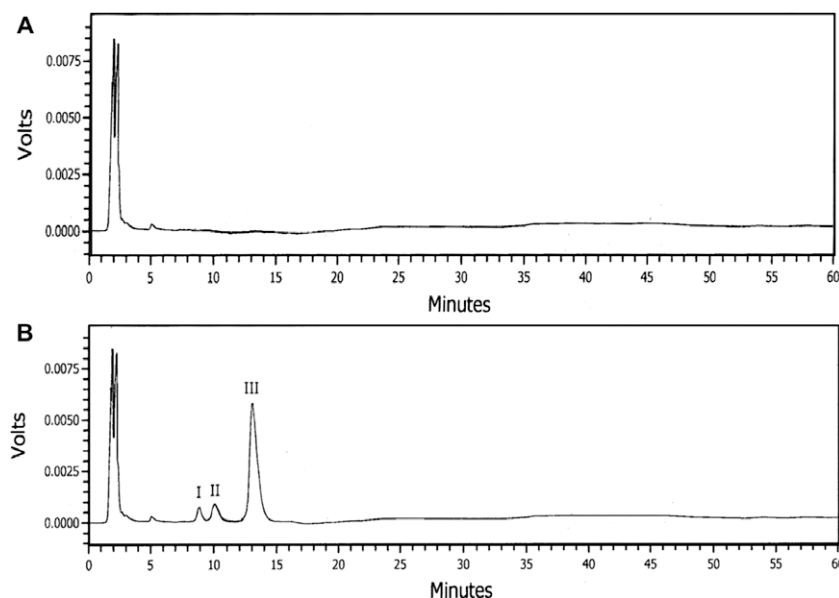


Figure 4 Chromatograms of (A) blank human plasma and (B) spiked with (I) S-(+)-arotinolol, (II) R-(–)-arotinolol, and (III) haloperidol (IS) using Chirobiotic V column (mobile phase: methanol:glacial acetic acid:triethylamine (100:0.020:0.030, v/v/v), flow rate: 1 ml/min, UV detection: 315 nm).

Table 4 Accuracy and precision data for arotinolol enantiomers in spiked plasma samples.

Analyte	Actual concentration (ng/ml)	Experimental concentration (ng/ml)	RSD (%) ^c	Error (%) ^d
Within-run ^a	80	82.49 ± 1.63	1.97	3.11
S-(+)-arotinolol	150	145.75 ± 8.08	5.54	-2.83
	300	293.53 ± 15.20	5.17	-2.15
R-(-)-arotinolol	80	81.76 ± 1.58	1.93	2.20
	150	145.61 ± 10.47	7.19	-2.92
	300	289.40 ± 16.73	5.77	-3.46
Between-run ^b	80	80.71 ± 4.64	5.74	0.88
S-(+)-arotinolol	150	147.15 ± 6.06	4.11	-1.90
	300	298.35 ± 16.67	5.58	-0.55
R-(-)-arotinolol	80	83.07 ± 3.98	4.79	3.83
	150	146.05 ± 5.69	3.89	-2.63
	300	293.58 ± 14.21	4.84	-2.14

^a Mean ± S.D. based on $n = 6$.^b Mean ± S.D. based on $n = 9$.^c Expressed as %RSD: (S.D./mean) × 100.^d Calculated as (experimental concentration-actual concentration/actual concentration) × 100.**Table 5** Determination of arotinolol enantiomers in pharmaceutical formulations by the proposed method.

Pharmaceutical preparation	Enantiomer	Nominal conc. (ng/ml)	Measured conc. (ng/ml)	Recovery (%)
Arotinolol tablet ^a	S-(+)-	100	98.4	98.4
		220	217.4	98.8
		340	335.2	98.6
Overall recovery (± SD)				98.60 ± 0.16
RSD (%)				0.16
	R-(-)-	100	101.8	101.8
		220	214.8	97.6
		340	335.4	98.6
Overall recovery (± SD)				99.30 ± 1.79
RSD (%)				1.80

^a Prepared in our Lab.

3.5. Limit of detection and limit of quantitation

The LOD and the LOQ in plasma sample as defined in Section 2 were 20 and 50 ng/ml for each enantiomer, respectively (Table 3).

3.6. Application to pharmaceutical formulation

The validity of the method developed here was applied to various concentrations taken from the pharmaceutical formulations for determining their content of arotinolol enantiomers. The values of the overall drug percentage recoveries and the %RSD value of S- and R-arotinolol enantiomers are presented in Table 5, indicating that these values are acceptable and the method is accurate and precise (Fig 5).

3.7. Selectivity

The analytical figures of merit for this method are shown in Table 1. Arotinolol enantiomers were well separated under the HPLC conditions applied. The drug was completely resolved from its degradation products. No interference was observed in drug free human plasma samples.

Excipients commonly coformulated with the studied drug such as magnesium stearate, cellulose, starch, calcium hydrogen phosphate, colloidal silicon dioxide and coloring agents, also did not interfere with the determination of arotinolol enantiomers, indicating the high selectivity of the method.

4. Conclusions

An enantioselective stability-indicating HPLC method was developed for the analysis of arotinolol in standard solution. The drug was found to degrade in alkaline, acidic, oxidative conditions and when exposed to heat. The drug was stable to sunlight. The method was highly selective, where degradation products and coformulated compounds did not interfere. The proposed method was successfully applied in human plasma and in pharmaceutical preparations. It was linear over the range of 50–400 ng/ml with a detection limit of 20 ng/ml for each enantiomer in spiked plasma sample.

The mean RSD of the results of within-day precision and accuracy of the drug were ≤7.1%. There was no significant difference between inter- and intra-day studies for each enan-

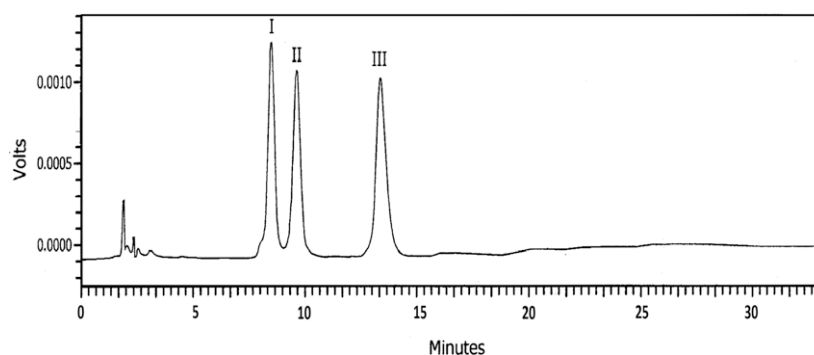


Figure 5 Chromatogram of (I) S-(+)-arotinolol (II) R-(-)-arotinolol recovered from tablets spiked with (III) haloperidol (IS) using Chirobiotic V column (mobile phase: methanol:glacial acetic acid:triethylamine (100:0.020:0.030, v/v/v), flow rate: 1 ml/min, UV detection: 315 nm).

tiomer which confirmed the reproducibility of the assay. The method was simple and suited to the routine control of the enantiomeric excess in the bulk drug.

Acknowledgements

The authors would like to thank Sumitomo Pharmaceutical Co. (Osaka, Japan) for their supply of S-(+)- and R-(-)-arotinolol.

References

- Aboul-Enein, H.Y., Ali, I., 2000. *Chromatographia* 52, 679.
- Aboul-Enein, H.Y., Ali, I., 2002. *Il Farmaco* 57, 513.
- Aboul-Enein, H.Y., Hefnawy, M.M., 2003. *Biomed. Chromatogr.* 17, 453.
- Ahuja, S., 1998. *Impurities Evaluation of Pharmaceuticals*. Marcel Dekker, Inc., New York, USA.
- Ahuja, S., Alsante, K.M., 2003. *Handbook of Isolation and Characterisation of Impurities in Pharmaceuticals*. Academic Press, San Diego, USA.
- Baaske, D.M., Demay, J.F., Latona, C.A., Mirmira, S., Sigvardson, K.W., 1996. *Am. J. Health-Syst. Pharm.* 53, 1701.
- Chirobiotic Handbook, 2004. *Advanced Separation Technology*, fifth ed. Whipppany, New York.
- Daraghme, N., Al-Omari, M., Badwan, A.A., Jaber, A.M., 2001. *J. Pharm. Biomed. Anal.* 25, 483.
- Duret, Ph., Foucault, A., Margraff, R., 2000. *J. Liq. Chromatogr. Relat. Technol.* 23, 295.
- Ekborg-Ott, K.H., Liu, Y., Armstrong, D.W., 1998. *Chirality* 10, 434.
- FDA, 1998. *Guidance for Industry: Impurities in Drug Product*, Draft Guidance. Center for Drug Evaluation and Research (CDER).
- Fried, K.M., Koch, P., Wainer, I.W., 1998. *Chirality* 10, 484.
- Hefnawy, M.M., 2002. *Chirality* 14, 67.
- ICH, 2003. *Stability testing of new drug substances and products Q1A (R2)*. In: *International Conference on Harmonization*. IFPMA, Geneva.
- Imai, Y., Watanabe, N., Hashimoto, J., Sakuma, H., Omata, K., Abe, K., 1995. *Eur. J. Clin. Pharmacol.* 48, 29.
- Moulin, A., Truer, D., Maillet, E., Dufour, A., 1992. *J. Liq. Chromatogr.* 15, 151.
- Pettersson, C., Persson, B., 1998. In: Katz, E., Eksteen, R., Schoenmakers, P., Miller, N. (Eds.), *Handbook of HPLC*. Marcel Dekker, New York, p. 669.
- Subramanian, G., 1994. In: Subramanian, G. (Ed.), *A Practical Approach to Chiral Separations by Liquid Chromatography*. VCH, Verlagsgesellschaft mbH, Weinheim, Germany.
- The United States Pharmacopeia, 2000. *United States Pharmacopeial Convention*, 24th ed. Rockville, MD, p. 2150.
- Török, G., Goetelen, L., Luyckx, R., Broeck, P.V., 2005. *J. Pharm. Biomed. Anal.* 39, 425.
- Van-Zwieten, P.A., 1993. *Drugs* 45, 509.